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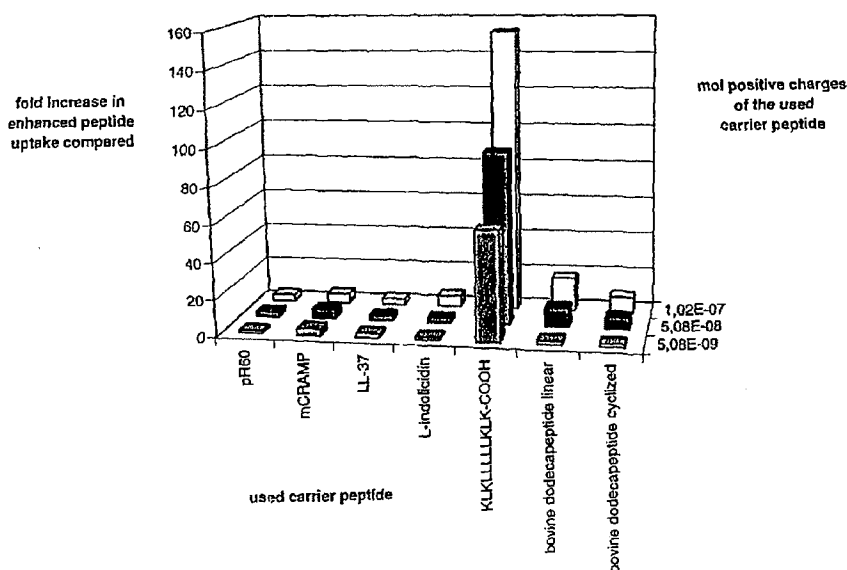
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(54) Title: VACCINE COMPOSITION



(57) Abstract: The invention relates to a vaccine which comprises at least one antigen and a peptide comprising a sequence $R_1\text{-XZXX}_N\text{XZX-R}_2$, whereby N is a whole number between 3 and 7, preferably 5, -X is a positively charged natural and/or non-natural amino acid residue, Z is an amino acid residue selected from the group consisting of L, V, I, F and/or W, and R_1 and R_2 are selected independently from the other from the group consisting of -H, -NH₂, -COCH₃, -COH, a peptide with up to 20 amino acid residues or a peptide reactive group or a peptide linker with or without a peptide; X-R₂ may also be an amide, ester or thioester of the C-terminal amino acid residue, as well as the use of said peptide for the preparation of an adjuvant for enhancing the immune response to at least one antigen.

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Vaccine composition

The present invention relates to vaccines comprising at least one antigen and an immunostimulating substance.

Host protection from invading pathogens involves cellular and humoral effectors and results from the concerted action of both non-adaptive (innate) and adaptive (acquired) immunity. The latter is based on specific immunological recognition mediated by receptors, is a recent acquisition of the immune system, and is present only in vertebrates. The former evolved before the development of adaptive immunity, consisting of a variety of cells and molecules distributed throughout the organism with the task of keeping potential pathogens under control (Boman, H. (2000)), (Zanetti, M. (1997)).

B and T lymphocytes are the mediators of acquired antigen-specific adaptive immunity, including the development of immunological memory, which is the main goal of creating a successful vaccine (Schijns, V. (2000)). Antigen presenting cells (APCs) are highly specialized cells that can process antigens and display their processed fragments on the cell surface together with molecules required for lymphocyte activation. This means that APCs are very important for the initiation of specific immune reactions. The main APCs for T lymphocyte activation are dendritic cells (DCs), macrophages, and B cells, whereas the main APCs for B cells are follicular dendritic cells. In general DCs are the most powerful APCs in terms of initiation of immune responses stimulating quiescent naive and memory B and T lymphocytes.

The natural task of APCs in the periphery (e.g. DCs or Langerhans cells) is to capture and process antigens, thereby being activated they start to express lymphocyte co-stimulatory molecules, migrate to lymphoid organs, secrete cytokines and present antigens to different populations of lymphocytes, initiating antigen-specific immune responses. They not only activate lymphocytes, under certain circumstances, they also tolerize T cells to antigens (Banchereau, J. (1998)).

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Antigen recognition by T lymphocytes is major histocompatibility complex (MHC)-restricted. A given T lymphocyte will recognize an antigen only when the peptide is bound to a particular MHC molecule. In general, T lymphocytes are stimulated only in the presence of self MHC molecules, and antigen is recognized only as peptides bound to self MHC molecules. MHC restriction defines T lymphocyte specificity in terms of the antigen recognized and in terms of the MHC molecule that binds its peptide fragment.

Intracellular and extracellular antigens present quite different challenges to the immune system, both in terms of recognition and of appropriate response. Presentation of antigens to T cells is mediated by two distinct classes of molecules - MHC class I (MHC-I) and MHC class II (MHC-II), which utilize distinct antigen processing pathways. Mainly one could distinguish between two major antigen processing pathways that have evolved. Peptides derived from intracellular antigens are presented to CD8⁺ T cells by MHC class I molecules, which are expressed on virtually all cells, while extracellular antigen-derived peptides are presented to CD4⁺ T cells by MHC-II molecules (Monaco, J. (1992); Harding, C. (1995)). However, there are certain exceptions to this dichotomy. Several studies have shown that peptides generated from endocytosed particulate or soluble proteins are presented on MHC-I molecules in macrophages as well as in dendritic cells (Harding, C. (1996); Brossart, P. (1997)). Therefore APCs like dendritic cells sitting in the periphery, exerting high potency to capture and process extracellular antigens and presenting them on MHC-I molecules to T lymphocytes are interesting targets in pulsing them extracellularly with antigens in vitro and in vivo.

The important and unique role of APCs, including stimulating activity on different types of leukocytes, is reflecting their central position as targets for appropriate strategies in developing successful vaccines. Theoretically one way to do so is to enhance or stimulate their natural task, the uptake of antigen(s). Once pulsed with the appropriate antigens the vaccine is directed against, APCs should start to process the taken up antigen(s), thereby being activated, expressing lymphocyte co-stimulatory molecules, migrating to lymphoid organs, secreting cytokines and presenting antigens to different populations of lymphocytes

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thereby initiating immune responses.

Activated T cells generally secrete a number of effector cytokines in a highly regulated fashion, e.g. interleukin 2 (IL-2), IL-4, IL-5, IL-10 and interferon- γ (IFN- γ). The functional detection of cytotoxic T lymphocyte responses to specific antigens (e.g. tumor antigens, in general antigens administered in a vaccine) is commonly monitored by an ELISpot assay (enzyme-linked immunospot assay), a technique analyzing cytokine production at the single cell level. In the present invention an ELISpot assay for the cellular immunity promoting cytokine IFN- γ was used to monitor successful peptide-specific T cell activation.

It has previously been shown that polycations efficiently enhance the uptake of MHC class I-matched peptides into tumor cells, a peptide or protein pulsing process which was called "TRANSloading" (Buschle, M. (1997)). Furthermore, we have shown that polycations are able to "TRANSload" peptides or proteins into antigen presenting cells in vivo as well as in vitro (Buschle, M. (1998)). In addition, co-injection of a mixture of poly-L-arginine or poly-L-lysine together with an appropriate peptide as a vaccine protects animals from tumor growth in mouse models (Schmidt, W. (1997)). This chemically defined vaccine is able to induce a high number of antigen/peptide-specific T cells. That was shown to be at least partly attributable to an enhanced uptake of peptides into APCs mediated by the polycation (Buschle, M. (1998)) indicating that APCs when pulsed in vivo with antigens can induce T cell-mediated immunity to the administered antigen.

As opposed to adaptive immunity, which is characterized by a highly specific but relatively slow response, innate immunity is based on effector mechanisms that are triggered by differences in the structure of microbial components relative to the host. These mechanisms can mount a fairly rapid initial response, which mainly leads to neutralization of the noxious agents. Reactions of innate immunity are the only defense strategy of lower phyla and have been retained in vertebrates as a first line host defense before the adaptive immune system is mobilized.

In higher vertebrates the effector cells of innate immunity are

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neutrophils, macrophages, and natural killer cells and probably also dendritic cells (Mizukawa, N. (1999)), whereas the humoral components in this pathway are the complement cascade and a variety of different binding proteins (Boman, H. (2000)).

A rapid and effective component of innate immunity is the production of a large variety of microbicidal peptides with a length of usually between about 12 and about one hundred amino acid residues. Several hundred different antimicrobial peptides have been isolated from a variety of organisms, ranging from sponges, insects to animals and humans, which points to a wide-spread distribution of these molecules. Antimicrobial peptides are also produced by bacteria as antagonistic substances against competing organisms.

In EP 0 905 141 A1 a peptide fragment of a limulus anti-LPS factor (LALF) having antiviral action is disclosed. This LALF peptide does not specifically enhance an immune response but enhances the non-specific defences of mononuclear cells and can also be used in a prophylactic way or further the peptide can also be administered topically to a wound site to stimulate an enhanced wound healing and repair.

Main sources of antimicrobial peptides are granules of neutrophils and epithelial cells lining the respiratory, gastro-intestinal and genitourinary tracts. In general they are found at those anatomical sites most exposed to microbial invasion, are secreted into internal body fluids or stored in cytoplasmic granules of professional phagocytes (neutrophils) (Ganz, T. (1997); Ganz, T. (1998); Ganz, T. (1999); Boman, H. (2000); Gudmundsson, GH. (1999)).

It has been shown previously (Austrian patent application A 1416/2000) that naturally occurring, cathelicidin-derived antimicrobial peptides or derivatives thereof have an immune response stimulating activity and therefore constitute highly effective adjuvants.

The aim of the present invention is to provide an adjuvant/"carrier peptide" that is able to strongly enhance the immune re-

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sponse to a specific co-administered antigen and therefore constitutes a highly effective adjuvant.

This object is solved by a vaccine which comprises at least one antigen and a peptide comprising a sequence $R_1\text{-XZXXZ}_N\text{XZX-R}_2$, whereby

- N is a whole number between 3 and 7, preferably 5,
- X is a positively charged natural and/or non-natural amino acid residue,
- Z is an amino acid residue selected from the group consisting of L, V, I, F and/or W, and
- R_1 and R_2 are selected independantly one from the other from the group consisting of -H, -NH_2 , -COCH_3 , -COH , a peptide with up to 20 amino acid residues or a peptide reactive group or a peptide linker with or without a peptide; X-R_2 may also be an amide, ester or thioester of the C-terminal amino acid residue.

Besides naturally occuring antimicrobial peptides, synthetic antimicrobial peptides have been produced and investigated. The synthetic antimicrobial peptide KLKLLLLLKLK-NH_2 was shown to have significant chemotherapeutic activity in Staphylococcus aureus-infected mice; human neutrophils were activated to produce the superoxide anion (O_2^-) via cell surface calreticulin. The exact number and position of K and L was found to be critical for the antimicrobial activity of the synthetic peptide (Nakajima, Y. (1997); Cho, J-H. (1999)).

It has now been surprisingly shown within the course of the present invention that peptides according to the present invention comprising a sequence $R_1\text{-XZXXZ}_N\text{XZX-R}_2$, whereby

- N is a whole number between 3 and 7, preferably 5,
- X is a positively charged natural and/or non-natural amino acid residue,
- Z is an amino acid residue selected from the group consisting of L, V, I, F and/or W, and
- R_1 and R_2 are selected independantly one from the other from the group consisting of -H, -NH_2 , -COCH_3 , -COH , a peptide with up to 20 amino acid residues or a peptide reactive group or a peptide linker with or without a peptide; X-R_2 may also be an

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amide or ester (or even thioester) of the C-terminal amino acid residue

(in the following termed as "peptides A") TRANSload antigenic peptides or proteins into APCs far more efficiently than known adjuvants, including naturally occurring antimicrobial peptides. They further have a strong immune response stimulating activity and therefore constitute highly effective adjuvants.

Preferably, the C-terminus is not modified (COOH or COO⁻), since this form is even better than the amidated form of the peptide.

In the scope of the present invention the sequence may be amidated at its carboxy-end or carry a further amino acid sequence, however; preferably the carboxy-end is free.

Furthermore, in the scope of the present invention, all the X comprised in the peptides alpha may represent the same amino acid residue. Preferably, however, in one peptide alpha X represents only one specific amino acid residue, e.g. either K or R, etc. The same can be applied with respect to Z: all the Z in the peptides alpha may be one single amino acid species or different amino acid species: e.g. either L or V etc.. This is especially the case for the Z_N-portion in the middle of the formula, which may be e.g. L₅ or L₃ as well as LVIFW, LILFLLIW, WIF, W₃L₂, and all other combinations of this motif, being between 3 and 7 amino acids at length, preferably from 4 to 6 amino acid residues, especially 5 amino acid residues. These residues are also preferred for the R₁ and R₂ portion (e.g. that more than 50%, preferably more than 80%, especially more than 90% of R₁ and/or R₂ are L, I, F, V and/or W, if R₁ and/or R₂ are peptides). Preferably R₁ and R₂ are the same, advantageously they are both H (i.e. free amino- or carboxy-termini).

Under the scope of the present invention the term "non-natural" comprises any amino acid residue which does not naturally occur and do not occur in natural proteins, respectively.

Peptide R₁-KLKL₅KLK-R₂ is specifically preferred, however also R₁-KIKL₅KIK-R₂, R₁-KVKL₅KVK-R₂, R₁-KPKL₅KVK-R₂, R₁-KLKL₆KLK-R₂, R₁-KWKW₅KLK-R₂, R₁-KWKWL₃WKWK-R₂, R₁-KLKL₄KLK-R₂ or permutations with

respect to positions of I, F, V, W and L are advantageous.

Of course, the vaccine may comprise two or more antigens depending on the desired immune response. The antigen(s) may also be modified so as to further enhance the immune response.

Preferably, proteins or peptides derived from viral or bacterial pathogens, from fungi or parasites, as well as tumor antigens (cancer vaccines) or antigens with a putative role in autoimmune disease are used as antigens (including derivatized antigens like glycosylated, lipidated, glycolipidated or hydroxylated antigens). Furthermore, carbohydrates, lipids or glycolipids may be used as antigens themselves. The derivatization process may include the purification of a specific protein or peptide from the pathogen, the inactivation of the pathogen as well as the proteolytic or chemical derivatization or stabilization of such a protein or peptide. Alternatively, also the pathogen itself may be used as an antigen. The antigens are preferably peptides or proteins, carbohydrates, lipids, glycolipids or mixtures thereof.

According to a preferred embodiment, T cell epitopes are used as antigens. Alternatively, a combination of T cell epitopes and B cell epitopes may also be preferred.

The antigens to be used in the present compositions are not critical. Also mixtures of different antigens are of course possible to be used according to the present invention. Preferably, proteins or peptides derived from a viral or a bacterial pathogen or from fungi or parasites are used as such antigens (including derivatized antigens or glycosylated or lipidated antigens or polysaccharides or lipids). Another preferred source of antigens are tumor antigens. Preferred pathogens are selected from human immunodeficiency virus (HIV), hepatitis A and B viruses, hepatitis C virus (HCV), rous sarcoma virus (RSV), Epstein Barr virus (EBV) Influenza virus, Rotavirus, Staphylococcus aureus, Chlamydia pneumonias, Chlamydia trachomatis, Mycobacterium tuberculosis, Streptococcus pneumonias, Bacillus anthracis, Vibrio cholerae, Plasmodium sp. (Pl. falciparum, Pl. vivax, etc.), Aspergillus sp. or Candida albicans. Antigens may also be molecules expressed by cancer cells (tumor antigens). The derivation proc-

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ess may include the purification of a specific protein from the pathogen/cancer cells, the inactivation of the pathogen as well as the proteolytic or chemical derivatization or stabilisation of such a protein. In the same way also tumor antigens (cancer vaccines) or autoimmune antigens may be used in the pharmaceutical composition according to the present invention. With such compositions a tumor vaccination or a treatment for autoimmune diseases may be performed.

In the case of peptide antigens the use of peptide mimotopes/agonists/superagonists/antagonists or peptides changed in certain positions without affecting the immunologic properties or non-peptide mimotopes/agonists/superagonists/antagonists is included in the current invention. Peptide antigens may also contain elongations either at the carboxy or at the amino terminus of the peptide antigen facilitating interaction with the polycationic compound(s) or the immunostimulatory compound(s). For the treatment of autoimmune diseases peptide antagonists may be applied.

Antigens may also be derivatized to include molecules enhancing antigen presentation and targeting of antigens to antigen presenting cells.

In one embodiment of the invention the pharmaceutical composition serves to confer tolerance to proteins or protein fragments and peptides which are involved in autoimmune diseases. Antigens used in this embodiments serve to tolerize the immune system or down-regulate immune responses against epitopes involved in autoimmune processes.

Preferably, the antigen is a peptide consisting of 5 to 60, preferably 6 to 30, especially 8 to 11, amino acid residues. Antigens of this length have been proven to be especially suitable for T cell activation. The antigens can further be coupled with a tail, e.g. according to A 657/2000, US 5,726,292 or WO98/01558.

The antigen may be mixed with the peptides of the present invention or otherwise specifically formulated e.g. as liposome, retard formulation, etc.. The antigen may also be covalently or non-covalently bound to the peptide according to the present in-

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vention. Preferably, the antigens are covalently bound to the peptide as R₁ or R₂ residues or to side chains of the amino acid residues of the peptide, especially to the K and R side chain.

The relative amounts of the ingredients of the present composition are highly dependent on the necessities of the individual composition. Preferably between 10 ng and 1 g of antigen and peptide alpha are applied. Preferred amounts of antigen/peptide alpha lie in the range of 0.1 to 1000 µg antigen per vaccination and 0.1 to 1000 µg peptide A. The composition according to the present invention may further contain auxiliary substances, such as buffers, salts, stabilizers, immunostimulants, antioxidants, etc., or other effective substances, such as antiinflammators or antinociceptive drugs.

The present compositions may be applied to a patient, e.g. a vaccination candidate, in efficient amounts, e.g. at weekly, bi-weekly or monthly intervals. Patients to be treated with the present composition may also be vaccinated repeatedly or only once. A preferred use of the present invention is the active immunization, especially of humans or animals without protection against the specific antigen.

The present composition may be applied subcutaneously, intramuscularly, rectally, intravenally, intradermally, intrapinnally, transdermally as well as by oral uptake.

Of course, the vaccine according to the present invention can comprise any further substance, as for example any other pharmaceutically acceptable carrier, etc. The vaccine according to the present invention may be formulated according to known methods, e.g. as i.v. vaccines, DNA vaccines, transdermal vaccines, topical vaccines, intranasal vaccines and as combination vaccines. The dosages may be selected by standard processes for vaccines which are improvements of known vaccines, however, a lower dosage than the known vaccine is possible for the same protection and therefore preferred.

Preferably, the vaccine is provided in a storage-stable form, e.g. lyophilized, optionally provided in combination with a suit-

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able reconstitution solution.

The amino acid residues according to the present invention may be D- or L-amino acids. Preferably, all or at least more than 80% of the residues belong to only one species (D or L). Most preferred, all amino acids in the peptide according to the present invention are of the same species (D or L). In some forms, the peptide according to the present invention may also comprise additional amino acid residues inserted in the sequence of peptide alpha, however, no A, G and T residues should be contained in the hydrophobic portion (Z, Z_N) of the peptide.

Preferably, in the peptide sequence X is an amino acid residue selected from the group consisting of K, R, ornithine and/or homoarginine. Again the X of one peptide alpha can be different amino acid residues selected from this group however, it is preferable that X is either K or R or ornithine or homoarginine in one peptide alpha.

According to a preferred embodiment of the present invention in the peptide sequence X is K. The peptide alpha comprising this amino acid as X has been shown to be particularly strong in inducing an immune response.

Preferably, in the peptide sequence Z is selected from the group consisting of L, V, I, F and/or W. As mentioned for X, also the Y can represent in one peptide alpha different amino acid residues. However, it is preferred that Z of one peptide alpha is only one amino acid residue, e.g. either L or V or I or F or W, whereby L and I residues are most preferred followed by F, followed by V and followed by W (L>I>F>V>W).

Still preferred, in the peptide alpha sequence Z is L (or I, especially L). Thereby, the peptide alpha is able to induce a particularly strong immune response.

Most preferred the peptide alpha is H-KLKL₁₁LL₁₁KLK-H. Of course, also the physiological form of this peptide (e.g. with a protonated N-terminus (NH₃⁺) and a deprotonated C-terminus (COO⁻)) shall be deemed to be incorporated in this formula (as for all

peptides according to the present invention).

According to a further advantageous embodiment, in the peptide sequence R_1 and/or R_2 is/are 10 to 20 amino acid residues. Thereby a peptide alpha is provided which has a length with which a particularly strong immune response is induced or improved.

According to an advantageous embodiment of the present invention, the amino acid residues of R_1 and/or R_2 are non-negatively charged amino acid residues.

Again, the amino acid residues can be natural and/or non-natural amino acid residues. By adding non-negatively charged amino acid residues at either one or both ends of the peptide alpha this peptide shows a strong capability for improving or inducing an immune response.

Preferably, R_1 and/or R_2 form a hydrophobic tail for the peptide A. Therefore, the amino acid residues of R_1 and/or R_2 are preferably selected from the group consisting of L, V, I, F and/or W. Still preferred, the amino acid residues of R_1 and/or R_2 are selected from the group consisting of L, I and/or F. Most preferred the additional amino acid residues are L. These peptides alpha show a particularly strong capability of inducing a higher immune response.

According to a preferred embodiment of the present invention the amino acid residues of R_1 and/or R_2 are positively charged natural and/or non natural amino acid residues. Preferably, the additional amino acid residues are selected from the group consisting of K, R, ornithine and/or homoarginine. Still preferred the amino acid residues of R_1 and/or R_2 are K. These peptides alpha also show a particularly good capability of improving the immune response.

It is preferred, that the amino acid residues of R_1 and/or R_2 are selected from the first group (consisting of L, V, I, F and/or W) or the second group (consisting of positively charged amino acid residues). However, it is also possible, that the amino acid residues of R_1 and/or R_2 are selected from both groups for one

single peptide alpha.

The peptide may be linked to the peptide alpha core of the present invention by normal peptide bounds or via peptide reactive groups or peptide linkers. Peptide reactive groups are chemical groups suitable for binding peptides or proteins. Therefore, the N- or C-terminus of the present peptide alpha may be chemically modified to comprise a chemical modification (e.g. iminothioane, 3-mercaptopropionyl, ...) allowing the covalent attachment of a peptide or an antigen, respectively. Alternatively, peptide alpha may comprise a suitable peptide linker, i.e. a linker molecule being able to form a link between the core peptide alpha (e.g. the peptide without R_1 and/or R_2) and e.g. an antigen linked or linkable thereto. The peptide according to the present invention may be present with or without the peptide/antigen being bound to the peptide reactive group and/or the peptide linker. Such chemical modifications and suitable peptide linkers are well available to the skilled man in the art.

Preferably, the vaccine comprises at least one further immune response stimulating substance. As immune response stimulating substance any substance or molecule can be used which is known to be active as an adjuvant. Such substances are disclosed in WO93/19768. Other substances may be e.g. polycations, as for example polylysine or polyarginine. Other adjuvants may be components in the form of particles, e.g. silicagel or dextran beads, which are sufficiently small so that they can enter into the cells. The addition of this further immune response stimulating substance will render the vaccine even more efficient.

Preferably the pharmaceutical composition according to the present invention, especially in the form of a vaccine, further comprises a polycationic polymer, preferably a polycationic peptide, especially polyarginine, polylysine or an antimicrobial peptide.

The polycationic compound(s) to be used according to the present invention may be any polycationic compound which shows the characteristic effect according to the WO 97/30721. Preferred polycationic compounds are selected from basic polypeptides, organic polycations, basic polyaminoacids or mixtures thereof. These

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polyaminoacids should have a chain length of at least 4 amino acid residues. Especially preferred are substances containing peptidic bounds, like polylysine, polyarginine and polypeptides containing more than 20%, especially more than 50% of basic amino acids in a range of more than 8, especially more than 20, amino acid residues or mixtures thereof. Other preferred polycations and their pharmaceutical compositions are described in WO 97/30721 (e.g. polyethyleneimine) and WO 99/38528. Preferably these polypeptides contain between 20 and 500 amino acid residues, especially between 30 and 200 residues.

These polycationic compounds may be produced chemically or recombinantly or may be derived from natural sources.

Cationic (poly)peptides may also be polycationic anti-bacterial microbial peptides. These (poly)peptides may be of prokaryotic or eukaryotic origin or may be produced chemically or recombinantly. Peptides may also belong to the class naturally occurring antimicrobial peptides. Such host defense peptides or defensives are also a preferred form of the polycationic polymer according to the present invention. Generally, a compound allowing as an end product activation (or down-regulation) of the adaptive immune system, preferably mediated by APCs (including dendritic cells) is used as polycationic polymer.

Especially preferred for use as polycationic substance in the present invention are cathelicidin derived antimicrobial peptides or derivatives thereof (A 1416/2000, incorporated herein by reference), especially antimicrobial peptides derived from mammalian cathelicidins, preferably from human, bovine or mouse.

Furthermore, also neuroactive compounds, such as (human) growth hormone (as described e.g. in WO01/24822) may be used as immunostimulants.

Polycationic compounds derived from natural sources include HIV-REV or HIV-TAT (derived cationic peptides, antennapedia peptides, chitosan or other derivatives of chitin) or other peptides derived from these peptides or proteins by biochemical or recombinant production. Other preferred polycationic compounds are

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cathelin or related or derived substances from cathelicidin, especially mouse, bovine or especially human cathelicidins and/or cathelicidins. Related or derived cathelicidin substances contain the whole or parts of the cathelicidin sequence with at least 15-20 amino acid residues. Derivations may include the substitution or modification of the natural amino acids by amino acids which are not among the 20 standard amino acids. Moreover, further cationic residues may be introduced into such cathelicidin molecules. These cathelicidin molecules are preferred to be combined with the antigen/vaccine composition according to the present invention. However, these cathelin molecules surprisingly have turned out to be also effective as an adjuvant for an antigen without the addition of further adjuvants. It is therefore possible to use such cathelicidin molecules as efficient adjuvants in vaccine formulations with or without further immunactivating substances.

Preferably, the immune response stimulating substance is a cytokine. Cytokines play an important role in activating and stimulating B cells, T cells and NK cells, macrophages, dendritic cells and various other cells participating in inducing immune responses. Any cytokine can be used which will additionally enhance the immune response to the antigen(s).

Preferably, the vaccine according to the present invention further comprises an immunostimulating/immunogenic nucleic acid, preferably an oligodeoxynucleotide containing deoxyinosine, an oligodeoxynucleotide containing deoxyuridine, an oligodeoxynucleotide containing a methylated or unmethylated CG motif or an inosine and cytidine containing nucleic acid molecule.

The immunogenic nucleic acids to be used according to the present invention can be of synthetic, prokaryotic and eukaryotic origin. In the case of eukaryotic origin, DNA should be derived from, based on the phylogenetic tree, less developed species (e.g. insects, but also others). In a preferred embodiment of the invention the immunogenic oligodeoxynucleotide (ODN) is a synthetically produced DNA-molecule or mixtures of such molecules. Derivates or modifications of ODNs such as thiophosphate substituted analogues (thiophosphate residues substitute for

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phosphate) as for example described in US patents US 5,723,335 and US 5,663,153, and other derivatives and modifications, which preferably stabilize the immunostimulatory composition(s) but do not change their immunological properties, are also included. A preferred sequence motif is a six base DNA motif containing an (unmethylated) CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines (5'-Pur-Pur-C-G-Pyr-Pyr-3'). The CpG motifs contained in the ODNs according to the present invention are more common in microbial than higher vertebrate DNA and display differences in the pattern of methylation. Surprisingly, sequences stimulating mouse APCs are not very efficient for human cells. Preferred palindromic or non-palindromic ODNs to be used according to the present invention are disclosed e.g. in Austrian Patent applications A 1973/2000, A 805/2001, EP 0 468 520 A2, WO 96/02555, WO 98/16247, WO 98/18810, WO 98/37919, WO 98/40100, WO 98/52581, WO 98/52962, WO 99/51259 and WO 99/56755 all incorporated herein by reference. Apart from stimulating the immune system certain ODNs are neutralizing some immune responses. These sequences are also included in the current invention, for example for applications for the treatment of autoimmune diseases. ODNs/DNAs may be produced chemically or recombinantly or may be derived from natural sources. Preferred natural sources are insects.

Alternatively, also nucleic acids based on inosine and cytidine (as e.g. described in the PCT/EP01/06437) or deoxynucleic acids containing deoxyinosine and/or deoxyuridine residues (described in the Austrian patent applications A 1973/2000 and A 805/2001, incorporated herein by reference) may preferably be used as immunostimulatory nucleic acids for the present invention.

Of course, also mixtures of different immunogenic nucleic acids may be used according to the present invention.

Another aspect of the present invention is the use of the peptide comprising the sequence R_1 -XZX_nXZX- R_2 (peptide A) as defined above for the preparation of an adjuvant for enhancing the immune response to at least one antigen.

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According to a preferred embodiment of the invention, the adjuvant is added to a vaccine. It is of course possible to administer the adjuvant directly to the mammal, e.g. preferably before the vaccination. It is, however, easier for the administration to add the adjuvant to a vaccine which is then administered to the mammal all at once.

According to a further aspect, the present invention relates to a method of vaccinating a mammal including humans against a specific antigen or a group of specific antigens, said method comprising the administration of an effective amount of a vaccine according to the present invention to said mammal, including humans, to be vaccinated. Alternatively, the method comprises administering an effective amount of an adjuvant comprising the peptide alpha as described above, after which a vaccine is administered.

The invention will be described in more detail by the following examples and figures, but the invention is of course not limited thereto.

Figure 1 shows the TRANSloading capacity of the (synthetic antimicrobial) peptide KLKLLLLLKLK (SEQ ID. No. 1) in comparison to diverse, previously described "carrier-peptides".

Figure 2 shows the effectivity of peptide variants according to the present invention compared to other peptides.

Figure 3 shows the amount of IFN- γ -producing cells in mice vaccinated with an antigenic peptide in combination with the (synthetic antimicrobial) peptide KLKLLLLLKLK.

EXAMPLES

Example 1

TRANSloading murine macrophages with a synthetic antimicrobial peptide as "carrier peptide"

To test if the (synthetic antimicrobial) peptide KLKLLLLLKLK is

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able to function as "carrier-peptide" for antigens, to TRANSload APCs in vitro, which means enhancing the antigen uptake into APCs, a fluorescently labelled peptide was used as antigenic peptide. It was mixed with diverse concentrations of KLKLLLLLLKLK and other previously described "carrier-peptides" as indicated.

To compare the efficiency of peptide delivery of these diverse "carrier-peptides", the amount of peptide uptake into APCs was monitored by incubating P388D1 cells (murine monocyte-macrophage antigen presenting cell line; purchased from ATCC (TIB-63)) for 1 h at 37°C with a constant amount of fluorescein-tagged peptide alone or in combination with diverse "carrier-peptides" at concentrations indicated. Before analysing the cells by flow cytometry, the cells were washed extensively to remove free peptide. The relative amount of fluorescein-tagged peptide taken up by the cells was measured by flow cytometry.

The antigenic peptide used is an influenza-haemagglutinin-derived MHC class I (Kd) binding peptide (Buschle, M. (1997)). 2µg of this antigenic peptide (FL-LFEAIEGFI) were mixed with 3 different amounts of each carrier peptide tested at concentrations representing 101.7, 50.9 and 5.09 nmol positive charges. (Figure 1 shows the fold increase in enhanced peptide uptake compared to peptide alone):

peptide FL-LFEAIEGFI mixed with

- (1) + poly-L-arginine (pR 60; 60 mer)
- (2) + murine cathelicidin-derived antimicrobial peptide (mCRAMP);
SEQ ID. No. 2
- (3) + LL-37; SEQ ID. No. 3
- (4) + L-indolicidin; SEQ ID. No 4
- (5) + KLKLLLLLLKLK (free C-terminus); SEQ ID. No. 1
- (6) + linear bovine dodecapeptide; SEQ ID. No. 5
- (7) + cyclized bovine dodecapeptide

Whereas fluorescence is known to be sparse in cells treated with peptide alone (as shown previously), intense fluorescence of "TRANSloaded" cells was especially found in cells which were TRANSloaded with the (synthetic antimicrobial) peptide KLKLLLLLLKLK as "carrier peptide", indicating that it is able to

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pulse APCs with an antigenic peptide very efficiently.

Example 2

TRANSloading murine macrophages with diverse synthetic antimicrobial peptides as "carrier peptides"

Diverse synthetic antimicrobial peptides of the sequence R_1 -XZZX_NXZX- R_2 , were tested to function as "carrier-peptide" for antigens, to TRANSload APCs in vitro, which means enhancing the antigen uptake into APCs. For that purpose, a fluorescent labelled peptide was used as antigenic peptide. It was mixed with diverse concentrations of peptides comprising a sequence R_1 -XZZX_NXZX- R_2 and other previously described "carrier-peptides" as indicated.

To compare the efficiency of peptide delivery of these diverse "carrier-peptides", the amount of peptide uptake into APCs was monitored by incubating P388D1 cells (murine monocyte-macrophage antigen presenting cell line; purchased from ATCC (TIB-63) for 1 h at 37°C with a constant amount of fluorescein-tagged peptide alone or in combination with diverse "carrier-peptides" at concentrations indicated. Before analysing the cells by flow cytometry, the cells were washed extensively to remove free peptide. The relative amount of fluorescein-tagged peptide taken up by the cells was measured by flow cytometry.

The antigenic peptide used is an influenza-haemagglutinin-derived MHC class I (Kd) binding peptide (Buschle, M. (1997)). 3µg of this antigenic peptide (FL-LFEAIEGFI) were mixed with 3 different amounts of each carrier peptide tested at concentrations representing 101.7, 50.9, and 5.09 nmol positive charges. (Figure 2 shows the fold increase in enhanced peptide uptake compared to peptide alone):

peptide FL-LFEAIEGFI mixed with

- (1) poly-L-arginine (60 mer)
- (2) Hp(2-20), a cecropin-like antibacterial peptide derived from the ribosomal protein L1 of Helicobacter pylori;
SEQ ID. No:6
- (3) LALF-peptide: SEQ ID No:7

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- (4) murine cathelicidine-derived antimicrobial peptide;
SEQ ID No:2
- (5) KAKAAAAAKAK-NH₂; SEQ ID. No:8
- (6) KGKGGGGGKKGK-NH₂; SEQ ID. No:9
- (7) KTKTTTTTKTK-NH₂; SEQ ID. No:10
- (8) KLKLVIFWKLK-NH₂; SEQ ID. No:11
- (9) KVKVVVVVKVK-NH₂; SEQ ID. No:12
- (10) KWKWWWWKWK-NH₂; SEQ ID. No:13
- (11) KFKFFFFFFKFK-NH₂; SEQ ID. No:14
- (12) RLKLLLLLKLR-NH₂; SEQ ID. No:15
- (13) RLRLLLLLRLR-NH₂; SEQ ID. No:16
- (14) KLKLLLLLKLK-NH₂; SEQ ID. No:17
- (15) KLKLLLLLKLK-COOH (free C-terminus); SEQ ID. No.1

Whereas fluorescence is known to be sparse in cells treated with peptide alone (as shown previously), intense fluorescence of "TRANSloaded" cells was especially found in cells which were TRANSloaded with the peptide comprising a sequence R₁-XZZX_NXZX-R₂ (including the above mentioned preferred embodiments) as "carrier peptide", indicating that the peptides according to the present invention are able to pulse APCs with an antigenic peptide very efficiently.

Example 3

Testing the ability to enhance the induction of peptide-specific T cell responses in vivo

For testing the ability of the (synthetic antimicrobial) peptide KLKLLLLLKLK to enhance the induction of peptide-specific T cell responses in vivo, groups of 4 mice (C57BL/6, female, 8 weeks of age, H-2b) were injected subcutaneously into the flank 3 times (days 0, 28, and 56), with an antigenic melanoma peptide (100µg) derived from TRP-2 (mouse tyrosinase related protein-2) alone or in combination with either poly-L-arginine or the (synthetic antimicrobial) peptide KLKLLLLLKLK as "carrier peptide". The amounts of the (synthetic antimicrobial) peptide KLKLLLLLKLK used represent four different amounts at concentrations representing

the equal amount (100µg) of poly-L-arginine in terms of µg, the equal (168µg), the double (336µg) and the triple (504µg) amount of poly-L-arginine in terms of positive charges. The groups of mice were injected as follows (amounts indicated/per mouse).

- (1) 100µg peptide
- (2) 100µg peptide + 100µg poly-L-arginine (pR 60)
- (3) 100µg peptide + 100µg KLKLLLLLLKLK
- (4) 100µg peptide + 168µg KLKLLLLLLKLK
- (5) 100µg peptide + 336µg KLKLLLLLLKLK
- (6) 100µg peptide + 504µg KLKLLLLLLKLK

12 days after the 3rd vaccination, draining (inguinal) lymph nodes were removed and lymph node cells (Figure 3) were activated *ex vivo* with TRP-2-derived (mouse tyrosinase related protein-2) peptide to determine IFN-γ-producing specific cells in an ELISpot assay (number of IFN-γ-ELISpots per million lymph node cells).

Figure 3 shows that injection of mice with peptide plus increasing amounts of KLKLLLLLLKLK resulted in many more IFN-γ-producing specific cells than injection of mice with peptide alone or in combination with poly-L-arginine. It has also been confirmed that the peptide KLKLLLLLLKLK does not elicit IFN-γ-producing peptide-specific T cells (as confirmed by ELISpot-assay), i.e. that only non KLKLLLLLLKLK specific T-cells have been obtained in the present experiments.

This example clearly demonstrates that the (synthetic anti-microbial) peptide KLKLLLLLLKLK enhances the induction of peptide-specific T cell responses *in vivo*.

In summary, the (synthetic antimicrobial) peptide KLKLLLLLLKLK showed a high "TRANSloading" and immunostimulating efficiency, indicating that peptides alpha are able to pulse APCs with antigenic peptides *in vitro* and *in vivo* very efficiently and are good adjuvants/"carrier-peptides" for antigenic peptides in inducing adaptive immune responses.

References:

Banchereau, et al. (1998), "Dendritic cells and the control of immunity", *Nature* 392(6673): 245-52.

Boman (2000), "Innate immunity and the normal microflora", *Immunol. Rev.* 173: 5-16.

Brossart, et al. (1997), "Presentation of exogenous protein antigens on major histocompatibility complex class I molecules by dendritic cells: pathway of presentation and regulation by cytokines", *Blood* 90(4): 1594-9.

Buschle, et al. (1998), "Chemically defined, cell-free cancer vaccines: use of tumor antigen-derived peptides or polypeptide proteins for vaccination", *Gene Therapy and molecular Biology* 1: 309-21.

Buschle, et al. (1997), "Transloading of tumor antigen-derived peptides into antigen-presenting cells", *Proc. Natl. Acad. Sci., USA* 94(7): 3256-61.

Cho, et al. (1999), "Activation of human neutrophils by a synthetic anti-microbial peptide, KLKLLLLLKLK-NH₂, via cell surface calreticulin", *Eur. J. Biochem.* 266: 878-85.

Ganz, et al. (1997), "Antimicrobial peptides of leukocytes", *Curr. Opin. Hematol.* 4(1): 53-8.

Ganz, T., (1998), "Antimicrobial peptides of vertebrates", *Curr. Opin. Immunol.* 10(1): 41-4.

Ganz, et al. (1999), "Antibiotic peptides from higher eukaryotes: biology and applications." *Mol. Med. Today* 5(7): 292-7.

Gudmundsson, et al. (1999), "Neutrophil antibacterial peptides, multifunctional effector molecules in the mammalian immune system", *J. Immunol. Methods* 232(1-2): 45-54.

Harding (1995), "Phagocytic processing of antigens for pre-sentation by MHC molecules", Trends in Cell Biology 5(3): 105-09.

Harding (1996), "Class I MHC presentation of exogenous antigens", J. Clin. Immunol. 16(2): 90-6.

Mizukawa, et al. (1999), "Presence of defensin in epithelial Langerhans cells adjacent to oral carcinomas and precancerous lesions", Anticancer Res. 19(4B): 2669-71.

Monaco (1992), "A molecular model of MHC class-I-restricted antigen processing", Immunol. Today 13(5): 173-9.

Nakajima, et al. (1997), "Chemotherapeutic activity of synthetic antimicrobial peptides: correlation between chemotherapeutic activity and neutrophil-activating activity", FEBS Lett. 415: 64-66.

Schijns (2000), "Immunological concepts of vaccine adjuvant activity", Curr. Opin. Immunol. 12(4): 456-63.

Schmidt, et al. (1997), "Cell-free tumor antigen peptide-based cancer vaccines", Proc. Natl. Acad. Sci., USA 94(7): 3262-7.

Zanetti, et al. (1997), "The cathelicidin family of antimicrobial peptide precursors: a component of the oxygen-independent defense mechanisms of neutrophils", Ann. N. Y. Acad. Sc. 832: 147-62.

Claims:

1. Vaccine, characterized in that it comprises at least one antigen and a peptide comprising a sequence $R_1\text{-XZ}_N\text{XZ}_N\text{-R}_2$, whereby
 - N is a whole number between 3 and 7, preferably 5,
 - X is a positively charged natural and/or non-natural amino acid residue,
 - Z is an amino acid residue selected from the group consisting of L, V, I, F and/or W, and
 - R_1 and R_2 are selected independantly one from the other from the group consisting of -H, -NH₂, -COCH₃, -COH, a peptide with up to 20 amino acid residues or a peptide reactive group or a peptide linker with or without a peptide; X- R_2 may also be an amide, ester or thioester of the C-terminal amino acid residue.
2. Vaccine according to claim 1, characterized in that in the peptide sequence X is an amino acid residue selected from the group consisting of K, R, ornithine and/or homoarginine.
3. Vaccine according to claim 2, characterized in that in the peptide sequence X is K.
4. Vaccine according to any one of claims 1 to 3, characterized in that in the peptide sequence Z is selected from the group consisting of L, I and/or F .
5. Vaccine according to claim 4, characterized in that in the peptide sequence Z is L.
6. Vaccine according to claim 1, characterized in that the peptide sequence is H-KLKLKLLKLLK-H.
7. Vaccine according to any one of claims 1 to 6, characterized in that in the peptide sequence R_1 and/or R_2 is/are 10 to 20 amino acid residues.
8. Vaccine according to claim 7, characterized in that the amino acid residues of R_1 and/or R_2 are non-negatively charged

amino acid residues.

9. Vaccine according to claim 8, characterized in that the amino acid residues of R_1 and/or R_2 are selected from the group consisting of L, V, I, F and/or W.

10. Vaccine according to claim 9, characterized in that the amino acid residues of R_1 and/or R_2 are selected from the group consisting of L, I and/or F.

11. Vaccine according to claim 10, characterized in that the amino acid residues of R_1 and/or R_2 are L.

12. Vaccine according to any one of claims 7 to 11, characterized in that the amino acid residues of R_1 and/or R_2 are positively charged natural and/or non-natural amino acid residues.

13. Vaccine according to claim 12, characterized in that the amino acid residues of R_1 and/or R_2 are selected from the group consisting of K, R, ornithine and/or homoarginine.

14. Vaccine according to claim 13, characterized in that the amino acid residues of R_1 and/or R_2 are K.

15. Vaccine according to any one of claims 1 to 14, characterized in that it comprises at least one further immune response stimulating substance.

16. Vaccine according to any one of claims 1 to 15, characterized in that it further comprises an immunostimulatory nucleic acid, preferably an oligodeoxynucleotide containing deoxyinosine, an oligodeoxynucleotide containing deoxyuridine, an oligodeoxynucleotide containing a CG motif or an inosine and cytidine containing nucleic acid molecule.

17. Vaccine according to any one of claims 1 to 16, characterized in that it further comprises a cytokine as immune response stimulating substance.

18. Vaccine according to any one of claims 1 to 17, character-

ized in that it further comprises a polycationic peptide, a neuroactive compound or a hormone with growth factor activity.

19. The use of the peptide comprising the sequence R_1 -XZZZ_NXZX- R_2 as defined in any one of claims 1 to 18 for the preparation of an adjuvant or carrier protein for enhancing the immune response to at least one antigen.

20. The use according to claim 19, characterized in that the adjuvant or carrier protein enhances the uptake of at least one antigen in antigen presenting cells (APC).

21. The use according to claim 19 or 20, characterized in that the adjuvant or carrier protein is added to a vaccine.

1/3

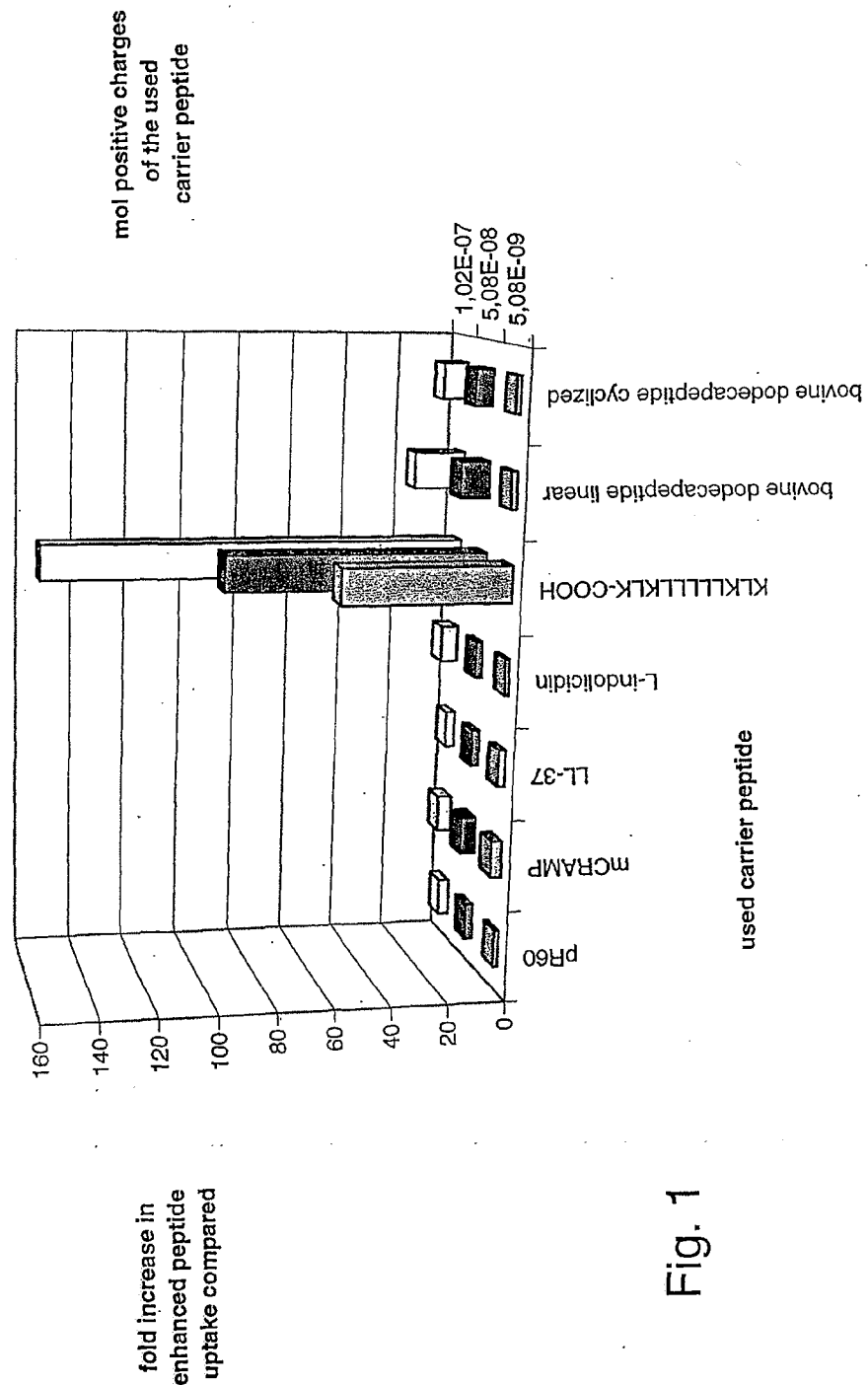
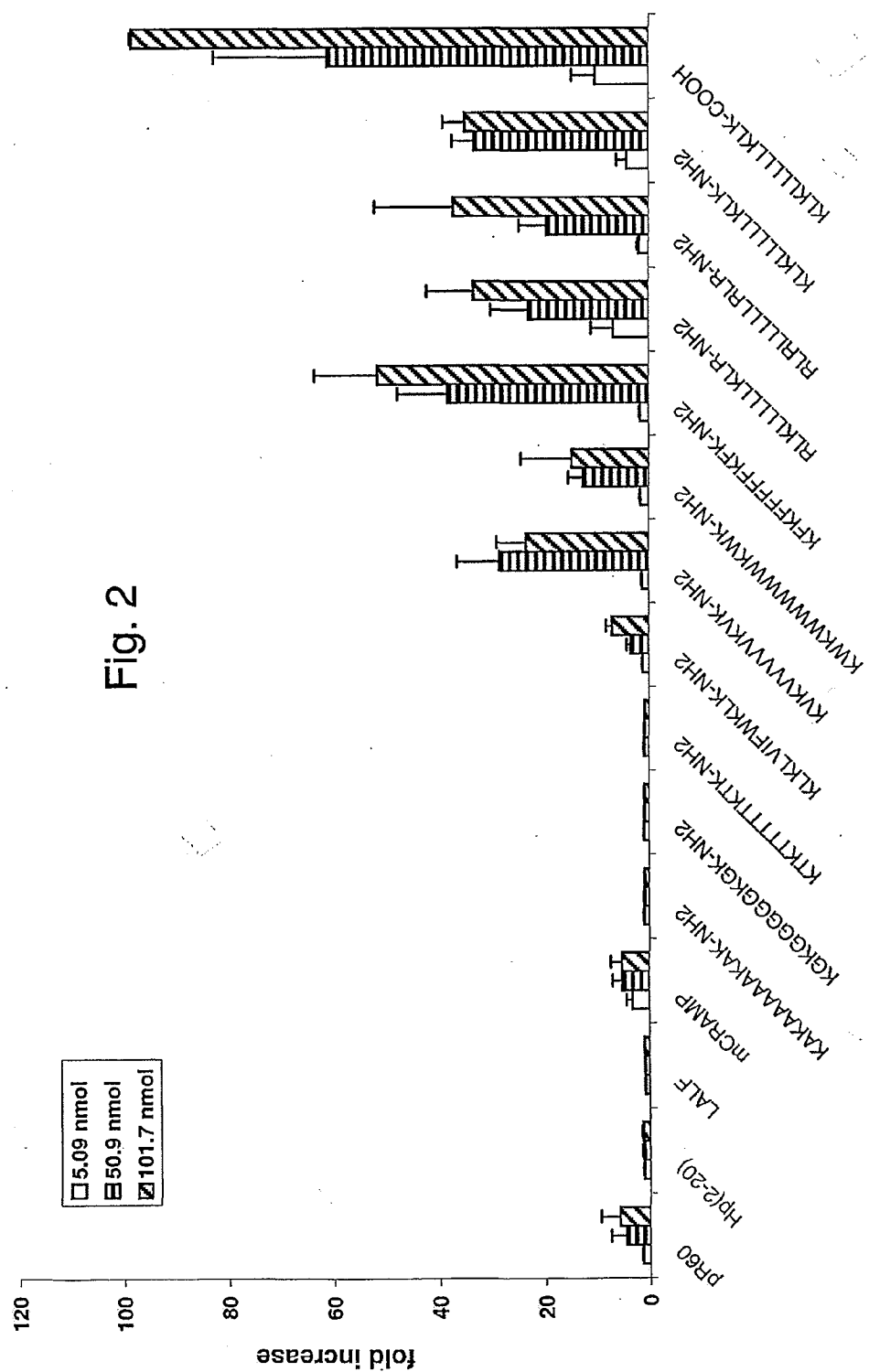
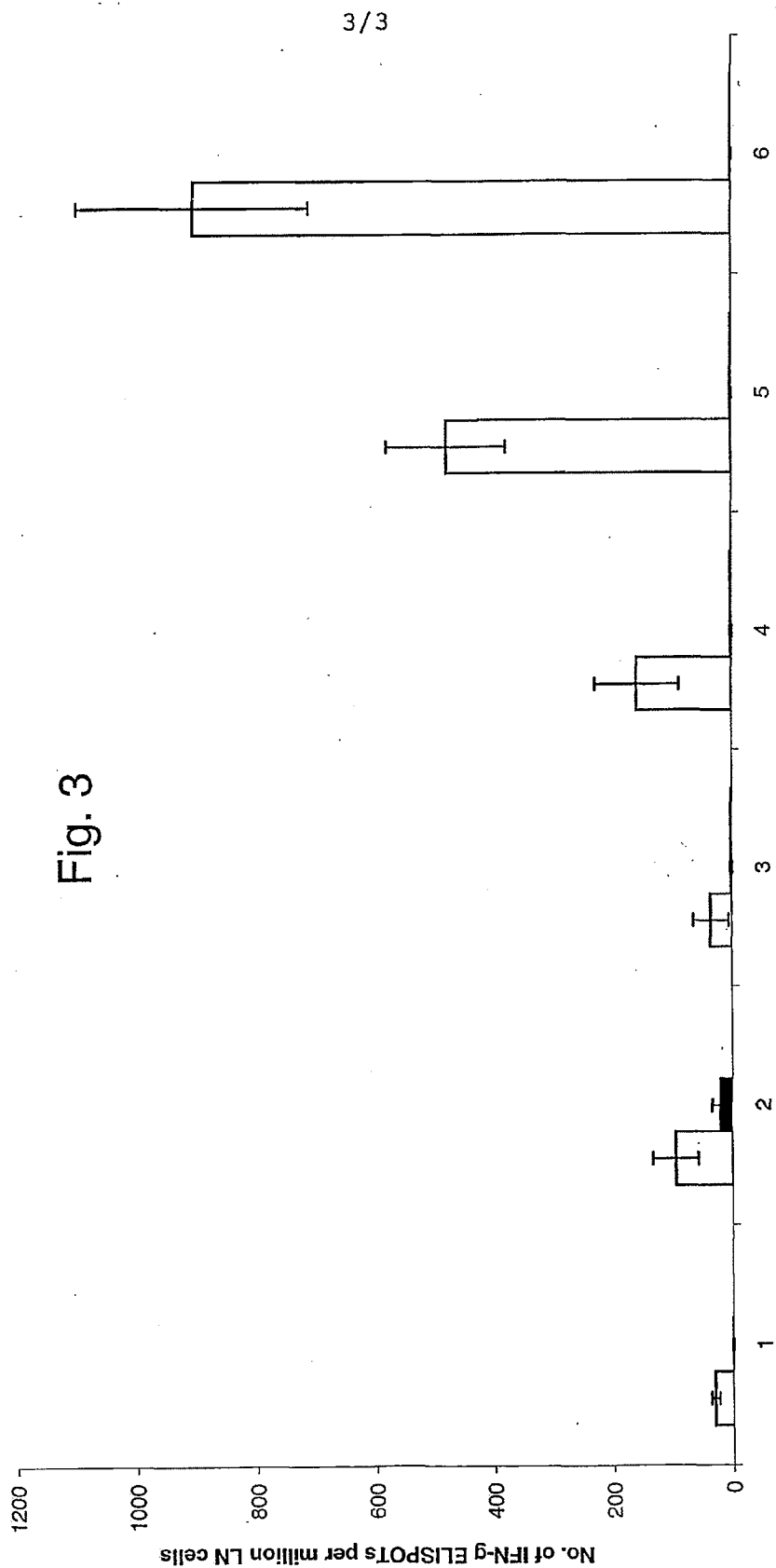


Fig. 1

Fig. 2





SEQUENCE LISTING

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/12041

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K39/00 A61K39/39 A61K38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, BIOSIS, CHEM ABS Data, EPO-Internal, WPI Data, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>BUSCHLE, MICHAEL (1) ET AL: "Development of defined, synthetic vaccines by in vivo charging of antigen presenting cells with antigen."</p> <p>JOURNAL OF INVESTIGATIVE DERMATOLOGY, (JAN., 2000) VOL. 114, NO. 1, PP. 235.</p> <p>MEETING INFO.: THE SIXTH INTERNATIONAL WORKSHOP ON LANGERHANS CELLS. NEW YORK, NEW YORK, USA OCTOBER 08-10, 1999 , XP000996450</p> <p>the whole document</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-6, 15-21

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

22 March 2002

Date of mailing of the international search report

05/04/2002

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INTERNATIONAL SEARCH REPORT

Interj nal Application No

PCT/EP 01/12041

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHO J H ET AL: "Activation of human neutrophils by a synthetic anti-microbial peptide, KKKLLLLLKK -NH ₂ , via cell surface calreticulin." EUROPEAN JOURNAL OF BIOCHEMISTRY, (1999 DEC) 266 (3) 878-85. , XP001056523 cited in the application the whole document ---	1-6, 15-21
A	ALVAREZ-BRAVO J ET AL: "Novel synthetic antimicrobial peptides effective against methicillin-resistant Staphylococcus aureus." BIOCHEMICAL JOURNAL, (1994 SEP 1) 302 (PT 2) 535-8. , XP001062698 the whole document -----	1-6, 15-21

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-6 (each partly), 7-14 (each as a whole) and 15-21 (each partly)

Claim 1 is directed to a vaccine comprising a peptide which is defined by a formula to which due to many optional features corresponds a very great number of embodiments. Nevertheless said broad formula is not reflected by any appropriate sequence in the sequence listing, which as such does not comply with the standard referred to in Rule 13ter(i) PCT. Moreover, a substantial support as required in Article 6 PCT appears to have been provided in the description as originally filed only for a number of peptides (see peptides 1 and 9-15 of Example 2; in said peptides R1 is -H only, R2 is either -NH₂ or COOH, "N" is always 5, and X is K or R) which together represent a very limited part of the embodiments encompassed by the formula of claim 1.

The afore-mentioned defects are such that it has not been possible to carry out a meaning search on the basis of the whole claimed subject-matter. The search has been limited to those embodiments of the subject-matter of claims 1-6 and 15-21 which are defined with a reference to a peptide as identified in the sequence listing using only the sequence identifiers (SEQ ID) NO. 1 and 12 to 17 to which correspond peptides 1 and 9-15 as referred to in Example 2.

Due to the limitation of the search, as the peptides referred to in the sequence listing using the sequence identifiers (SEQ ID) NO. 1 and 12 to 17 have no R1 or R2 group being a peptide, the subject-matter of claims 7-14 was not searched at all.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.